2513-Pos Board B205
The Transportation Potential of Human Serum Albumin for miR106A
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Genetic therapy using microRNA in sequence knock-downs can benefit from a vector delivery system to effectively deliver targeted therapy in vivo. RNAi (RNA interference) mechanisms use splitting of a double-stranded miRNA to a single-strand that down-regulates genetic expression. Human serum albumin (HSA), a blood plasma transport protein, was selected for investigation as a possible transport of single- or double-strand miRNA. The binding effects of a single or double-stranded mimic miRNA, miR-106-A, with HSA was studied through the use of time-resolved fluorescence anisotropy decay and FRET, using covalent linking of the dye Atto 390 to the 3' OH ends of the mimic RNA and Atto 488 to the N-terminus of HSA. Single-stranded RNA was achieved by heat denaturation and subsequent rapid annealing in the HSA solution to prevent double-stranding re-association. The results show that the ionic strength of the buffer has a strong effect on binding and that at physiological conditions using PBS buffer and 37 °C incubation, HSA does weakly bind the double-stranded RNA, but not the single-stranded form.

2514-Pos Board B206
Unsaturated Fatty Acid Regulation of AraC/XylS Transcription Factors
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Members of the AraC/XylS family of transcription factors play key roles in regulating virulence in a variety of pathogenic bacteria, but to date the structures of only 4 AraC/XylS family members have been solved. In addition to a lack of structural data, for many of the family members, ligands have yet to be determined. Based on previous structural data obtained in our lab indicating that an unsaturated fatty acid (UFA) was the ligand for the AraC/Xyls family member ToxT, we set out to determine if the activities of other members of this family could be regulated by UFAs. We have used a combination of computational and biochemical methods to study the effect of UFAs on the activity of VirF of Yersinia enterocolitica and Rns of enterotoxigenic E. coli. Here we describe preliminary findings, supporting our hypothesis that these AraC/Xyls family members are repressed by fatty acids. Additionally we show progress towards producing a fifth high resolution structure of an Arac/XylS family protein.

2515-Pos Board B207
Mechanistic Diversity in DNA Site Discrimination by Structurally Homologous ETS-Family Transcription Factors
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Eukaryotic transcription factors are characterized by large families that share structurally homologous DNA-binding domains and similar DNA site preferences. The molecular mechanism by which these functionally non-interchangeable homologs achieve specificity remains poorly known. The ETS-family of transcription factors represent an excellent model system that embodies this ''specificity conundrum.'' The 28 human ETS paralogs share a structurally conserved DNA-binding domain (known as the ETS domain) and recognize sites harboring a central 5'-GGAA/T-3' consensus. Although ETS domains are highly conserved structurally, they share low amino acid homology. We hypothesize that this apparent sequence space encodes divergent physicochemical properties that confers specificity to site discrimination at the protein-DNA level. We compared the solution properties of site discrimination by the ETS domains of PU.1 and Ets-1, two highly structurally conserved by sequence-divergent proteins. The proteins exhibit profound differences in interfacial hydration: whereas Ets-1 is weakly sensitive to perturbation in water activity, PU.1 is strongly destabilized by osmotic stress. The contrasting thermodynamics and kinetics of site recognition by PU.1 and Ets-1 indicate significant differences in their mechanisms of selectivity. Specifically, PU.1 extensively integrates structural water into high-affinity binding, a feature that renders site specificity by PU.1 highly sensitive to the solution osmotic status. PU.1 is expressed only in immune and closely-related cell lineages, all of which develop and function in osmotically variable environments. PU.1 and Ets-1 direct distinct responses in cells in which they are co-expressed. The strong sensitivity to osmotic stress in sequence discrimination by which PU.1 may represent a mechanism by which it functions distinctly from other ETS members such as Ets-1 in cells that must adapt to physiological osmotic stress.

2516-Pos Board B208
Small-Angle X-Ray Scattering Study for Conformational Changes of Unusual Transcription Factor at the Operator
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The chimeric transcription factors of the GntR family in bacteria contain a N-terminal winged helix-turn-helix (WHTH) DNA-binding domain and a C-terminal effector-binding/oligomerisation (EB/O) domain. The GntR family regulator GabR controls the expression of genes that are essential for glutamic acid metabolism. It represents an unusual transcription factor in that its EB/O domain is homologous to type I aminotransferases and is thought to form a head-to-tail dimer. Simultaneous binding of effector molecules such gamma-aminobutyric acid (GABA) and the co-enzyme pyridoxal-5'-phosphate (PLP) lead to activation of gene expression. This regulation is expected to involve conformational changes and/or changes in the number of transcription factors bound to DNA. Here we present the effect of such conformational change on small-angle X-ray scattering (SAXS) data, furthering the understanding of the linkage between the structure, dynamics, and function of GabR at the operator. The scattering observations are quantitatively investigated with respect to the average spatial extent of structural variations occurring in this transcription factor under diverse experimental conditions including scattering of the protein alone. The analyses of scattering data support that the spatial structural extent of this transcription factor at the operator is significant.

2517-Pos Board B209
Fidelity Control of a Non-Proofreading Polymerase Studied from Kinetics to Molecular Dynamics
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Polymerases catalyze template-based polymerization in gene replication and transcription. It is essential for the polymerase to achieve sufficiently high fidelity at sufficiently high speed. We investigated kinetically how polymerases select nucleotides efficiently under energy constraints. We noticed that initial screening is indispensable for lowering error rates without lowering much the polymerization speed. Still, multiple checkpoints seem to be necessary for the fidelity requirement overall. To see how exactly the nucleotide selection proceeds, we studied a single-subunit T7 RNA polymerase in molecular dynamics details. We found that substantial nucleotide selection happens prior to full insertion of the nucleotide or formation of the Watson-Crick base pairing. The initial selection replies primarily on electrostatic screening to destabilize a wrong nucleotide relative to the right one at the pre-insertion site. A highly conserved tyrosine can detect the nucleotide identity upon the pre-insertion, and assists the selection through 'gating' during the nucleotide insertion. In particular, the tyrosine residue differentiates dNTP from rNTP by favorably associating with dNTP but not rNTP. The mutation of this residue to phenylalanine loses this differentiation. That explains why the mutant was experimentally detected as not only an RNA polymerase, but also a DNA polymerase.

2518-Pos Board B210
Genome-Wide Organization of Eukaryotic Pre-Initiation Complex is Influenced by Non-Consensus Protein-DNA Binding
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Genome-wide binding preferences of the key components of eukaryotic preinitiation complex (PIC) have been recently measured at high resolution in Saccharomyces cerevisiae by Rhee and Pugh. However, the rules determining the PIC binding specificity remain poorly understood. In this study, we show that nonconsensus protein-DNA binding significantly influences PIC binding preferences. We estimate that such nonconsensus binding contributes statistically at least 2-3 kcal/mol (on average) of additional attractive free energy per protein per core-promoter region. The predicted attractive effect is particularly strong at repeated poly(dA:dT) and poly(dC:dG) tracts. Overall, the computed free-energy landscape of nonconsensus protein-DNA binding shows strong correlation with the measured genome-wide PIC occupancy. Remarkably, statistical PIC preferences of binding to both TFIID-dominated and SAGA-dominated genes correlate with the nonconsensus free-energy landscape, yet these two groups of genes are distinguishable based on the average free-energy profiles. We suggest that the predicted nonconsensus binding mechanism provides a genome-wide background for specific promoter elements, such as transcription-factor binding sites, TATA-like elements, and